

## PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING  
OF A CHANGE(PCT Rule 92bis.1 and  
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

PARK, A.J.  
6th Floor  
Huddart Parker Building  
One Post Square  
P.O. Box 949  
Wellington  
New Zealand

RECEIVED  
NOV - 8 2002  
TC 1700 MAIL ROOM

Date of mailing (day/month/year)

20 September 2002 (20.09.02)

Applicant's or agent's file reference

T/JP/U246WO

## IMPORTANT NOTIFICATION

International application No.

PCT/NZ00/00001

International filing date (day/month/year)

10 January 2000 (10.01.00)

1. The following indications appeared on record concerning:

☐

the applicant

☐

the inventor

☒

the agent

☐

the common representative

Name and Address

PIPER, James, William  
Pipers  
Unicorn House  
300A Richmond Road  
Grey Lynn  
Auckland 1002  
New Zealand

State of Nationality

State of Residence

Telephone No.

64 9 378 1861

Facsimile No.

64 9 378 1864

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☒

the person

☒

the name

☒

the address

☐

the nationality

☐

the residence

Name and Address

PARK, A.J.  
6th Floor  
Huddart Parker Building  
One Post Square  
P.O. Box 949  
Wellington  
New Zealand

State of Nationality

State of Residence

Telephone No.

64 4 473 8278

Facsimile No.

64 4 472 3358

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒

the receiving Office

☐

the International Searching Authority

☐

the International Preliminary Examining Authority

☐

the designated Offices concerned

☒

the elected Offices concerned

☐

other:

The International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.1435

Authorized officer

Kiwa MPAY

Telephone No.: (41-22) 338.83.38

## PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING  
OF A CHANGE(PCT Rule 92bis.1 and  
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

PIPER, James, William  
Pipers  
Unicorn House  
300A Richmond Road  
Grey Lynn  
Auckland 1002  
NOUVELLE-ZÉLANDE

Date of mailing (day/month/year) 07 February 2002 (07.02.02)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference T/JP/U246WO	
International application No. PCT/NZ00/00001	International filing date (day/month/year) 10 January 2000 (10.01.00)

1. The following indications appeared on record concerning:		
<input checked="" type="checkbox"/> the applicant	<input type="checkbox"/> the inventor	<input type="checkbox"/> the agent
<input type="checkbox"/> the common representative		
Name and Address BETA NUTRITION LIMITED 21 Cumming Road Oakford, W.A. 6205 Australia	State of Nationality AU	State of Residence AU
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	
2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:		
<input checked="" type="checkbox"/> the person	<input checked="" type="checkbox"/> the name	<input checked="" type="checkbox"/> the address
<input type="checkbox"/> the nationality		
<input type="checkbox"/> the residence		
Name and Address OXITENE PTY LIMITED Level 4 65 Berry Street North Sydney, NSW 2059 Australia	State of Nationality AU	State of Residence AU
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	
3. Further observations, if necessary: <b>The applicant indicated in Box 1 has assigned his rights for all designated States except US to the applicant indicated in Box 2. KEATING, Peter, James remains the sole applicant/inventor for US only.</b>		
4. A copy of this notification has been sent to:		
<input checked="" type="checkbox"/> the receiving Office	<input type="checkbox"/> the designated Offices concerned	
<input type="checkbox"/> the International Searching Authority	<input checked="" type="checkbox"/> the elected Offices concerned	
<input type="checkbox"/> the International Preliminary Examining Authority	<input type="checkbox"/> other:	

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer François BAECHLER Telephone No.: (41-22) 338.83.38
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## PATENT COOPERATION TREATY

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NOTIFICATION OF THE RECORDING  
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Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

PIPER, James, William  
Pipers  
Unicorn House  
300A Richmond Road  
Grey Lynn  
Auckland 1002  
NOUVELLE-ZÉLANDE

Date of mailing (day/month/year)  
07 February 2002 (07.02.02)

Applicant's or agent's file reference  
T/JP/U246WO

International application No.  
PCT/NZ00/00001

## IMPORTANT NOTIFICATION

International filing date (day/month/year)  
10 January 2000 (10.01.00)

## 1. The following indications appeared on record concerning:

☒ the applicant ☐ the inventor ☐ the agent ☐ the common representative

## Name and Address

PIPER, James, William  
90 Bassett Road  
Remuera  
Auckland 1005  
New Zealand

## State of Nationality

NZ

## State of Residence

NZ

Telephone No.

Facsimile No.

Teleprinter No.

## 2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☐ the address ☐ the nationality ☐ the residence

## Name and Address

## State of Nationality

## State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

## 3. Further observations, if necessary:

The applicant indicated above has been removed from our records.

## 4. A copy of this notification has been sent to:

☒ the receiving Office ☐ the designated Offices concerned  
☐ the International Searching Authority ☒ the elected Offices concerned  
☐ the International Preliminary Examining Authority ☒ other: PIPER, James, William

The International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

François BAECHLER

Telephone No.: (41-22) 338.83.38

## PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING  
OF A CHANGE(PCT Rule 92bis.1 and  
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

PIPER, James, William  
Pipers  
Unicorn House  
300A Richmond Road  
Grey Lynn  
Auckland 1002  
NOUVELLE-ZÉLANDE

Date of mailing (day/month/year) 12 March 2002 (12.03.02)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference T/JP/U246WO	
International application No. PCT/NZ00/00001	International filing date (day/month/year) 10 January 2000 (10.01.00)

1. The following indications appeared on record concerning:		
<input checked="" type="checkbox"/> the applicant	<input type="checkbox"/> the inventor	<input type="checkbox"/> the agent <input type="checkbox"/> the common representative
Name and Address OXITENE PTY LIMITED Level 4 65 Berry Street North Sydney, NSW 2059 Australia	State of Nationality AU	State of Residence AU
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	
2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:		
<input checked="" type="checkbox"/> the person	<input checked="" type="checkbox"/> the name	<input checked="" type="checkbox"/> the address <input checked="" type="checkbox"/> the nationality <input checked="" type="checkbox"/> the residence
Name and Address BETA CAROTENE INVESTMENTS LIMITED Level 14, Tower 2 The Shortland Centre 55-65 Shortland Street Auckland City New Zealand	State of Nationality NZ	State of Residence NZ
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	
3. Further observations, if necessary:		
4. A copy of this notification has been sent to:		
<input checked="" type="checkbox"/> the receiving Office	<input type="checkbox"/> the designated Offices concerned	
<input type="checkbox"/> the International Searching Authority	<input checked="" type="checkbox"/> the elected Offices concerned	
<input type="checkbox"/> the International Preliminary Examining Authority	<input type="checkbox"/> other:	

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer François BAECHLER Telephone No.: (41-22) 338.83.38
---	---

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To:

PIPER, James, William  
Pipers  
Unicorn House  
300A Richmond Road  
Grey Lynn  
Auckland 1002  
NOUVELLE-ZÉLANDE

RECEIVED

JAN 02 2002

Technology Center 2100

Date of mailing (day/month/year) 23 October 2001 (23.10.01)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference T/JP/U246WO	
International application No. PCT/NZ00/00001	International filing date (day/month/year) 10 January 2000 (10.01.00)

## 1. The following indications appeared on record concerning:

☐ the applicant ☐ the inventor ☒ the agent ☐ the common representative

Name and Address PIPER, James, William Pipers 16 Byron Avenue Takapuna, P.O. Box 33-1153 North Shore City Auckland New Zealand	State of Nationality	State of Residence
	Telephone No. 64 9 378 1861	
	Facsimile No. 64 9 378 1864	
	Teleprinter No.	

## 2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address PIPER, James, William Pipers Unicorn House 300A Richmond Road Grey Lynn Auckland 1002 New Zealand	State of Nationality	State of Residence
	Telephone No. 64 9 378 1861	
	Facsimile No. 64 9 378 1864	
	Teleprinter No.	

## 3. Further observations, if necessary:

The indication of a new address of the agent on the Demand (Form PCT/IPEA/401) has been considered a request for recording a change under Rule 92bis. In case of disagreement, the International Bureau should be notified immediately.

## 4. A copy of this notification has been sent to:

☒ the receiving Office ☐ the designated Offices concerned  
☐ the International Searching Authority ☒ the elected Offices concerned  
☒ the International Preliminary Examining Authority ☐ other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer François BAECHLER Telephone No.: (41-22) 338.83.38
---	---

**PCT**

(PCT Rule 61.2)

To:

**Commissioner  
US Department of Commerce  
United States Patent and Trademark  
Office, PCT  
2011 South Clark Place Room  
CP2/5C24  
Arlington, VA 22202  
ETATS-UNIS D'AMERIQUE**  
**in its capacity as elected Office**

<b>Date of mailing (day/month/year)</b> 23 October 2001 (23.10.01)	<b>ETATS-UNIS D'AMERIQUE</b> in its capacity as elected Office
<b>International application No.</b> PCT/NZ00/00001	<b>Applicant's or agent's file reference</b> T/JP/U246WO
<b>International filing date (day/month/year)</b> 10 January 2000 (10.01.00)	<b>Priority date (day/month/year)</b>
<b>Applicant</b> KEATING, Peter, James	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:

06 August 2001 (06.08.01)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was ☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

<p><b>The International Bureau of WIPO</b>  <b>34, chemin des Colombettes</b>  <b>1211 Geneva 20, Switzerland</b></p> <p>Facsimile No.: (41-22) 740.14.35</p>	<p>Authorized officer</p> <p><b>François BAECHLER</b></p> <p>Telephone No.: (41-22) 338.83.38</p>
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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

REC'D 21 AUG 2001

WIPO

PCT

Applicant's or agent's file reference T/JP/U246WO	<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).	
International Application No. <b>PCT/NZ00/00001</b>	International Filing Date (day/month/year) 10 January 2000	Priority Date (day/month/year) 10 January 2000
International Patent Classification (IPC) or national classification and IPC Int. Cl. <sup>7</sup> B01D 15/02, B01J 8/20, C07C 403/24		
Applicant [BETA NUTRITION LIMITED et al] <i>Keating, Peter, James</i>		

1.	This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.																
2.	<p>This REPORT consists of a total of 3 sheets, including this cover sheet.</p> <p><input type="checkbox"/> This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of sheet(s).</p>																
3.	<p>This report contains indications relating to the following items:</p> <table border="0"> <tr> <td>I</td> <td><input checked="" type="checkbox"/> Basis of the report</td> </tr> <tr> <td>II</td> <td><input type="checkbox"/> Priority</td> </tr> <tr> <td>III</td> <td><input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</td> </tr> <tr> <td>IV</td> <td><input type="checkbox"/> Lack of unity of invention</td> </tr> <tr> <td>V</td> <td><input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</td> </tr> <tr> <td>VI</td> <td><input type="checkbox"/> Certain documents cited</td> </tr> <tr> <td>VII</td> <td><input type="checkbox"/> Certain defects in the international application</td> </tr> <tr> <td>VIII</td> <td><input type="checkbox"/> Certain observations on the international application</td> </tr> </table>	I	<input checked="" type="checkbox"/> Basis of the report	II	<input type="checkbox"/> Priority	III	<input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability	IV	<input type="checkbox"/> Lack of unity of invention	V	<input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement	VI	<input type="checkbox"/> Certain documents cited	VII	<input type="checkbox"/> Certain defects in the international application	VIII	<input type="checkbox"/> Certain observations on the international application
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VI	<input type="checkbox"/> Certain documents cited																
VII	<input type="checkbox"/> Certain defects in the international application																
VIII	<input type="checkbox"/> Certain observations on the international application																

Date of submission of the demand 6 August 2001	Date of completion of the report 9 August 2001
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustalia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer  <b>G. Carter</b> Telephone No. (02) 62832154

**I. Basis of the report**

## 1. With regard to the elements of the international application:\*

- ☒ the international application as originally filed.
- ☐ the description,        pages , as originally filed,  
    pages , filed with the demand,  
    pages , received on    with the letter of
- ☐ the claims,                pages , as originally filed,  
    pages , as amended (together with any statement) under Article 19,  
    pages , filed with the demand,  
    pages , received on    with the letter of
- ☐ the drawings,            pages , as originally filed,  
    pages , filed with the demand,  
    pages , received on    with the letter of
- ☐ the sequence listing part of the description:  
    pages , as originally filed  
    pages , filed with the demand  
    pages , received on    with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. ☐ The amendments have resulted in the cancellation of:

- ☐ the description,        pages
- ☐ the claims,            Nos.
- ☐ the drawings,        sheets/fig.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).\*\*

\* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

\*\* Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report



**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement****1. Statement**

Novelty (N)	Claims 1-14	YES
	Claims	NO
Inventive step (IS)	Claims 1-14	YES
	Claims	NO
Industrial applicability (IA)	Claims 1-14	YES
	Claims	NO

**2. Citations and explanations (Rule 70.7)**

WO 92/198237

US 4284511

DE 19638004

None of the citations disclose a method of removing fat soluble compounds from aqueous solutions using a fluidised bed of crystalline metallic particles as claimed.

Art Unit: 1724

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 16-25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Curtain et al. (U.S. Patent No. 4,554,390) in view of Weitzen et al. (U.S. Patent No. 4,284,511). Curtain et al. discloses extracting a fat soluble compound, such as beta-carotene, from an aqueous brine solution containing *Dunaliella salina* with crystalline metallic ore particles, such as magnetite; washing the resultant complex; and recovering the beta-carotene from the wash solution (see Example 2). This reference further teaches that the algae containing the beta-carotene is "adsorbed" onto the magnetite (see col. 2, line 60). Accordingly, this primary reference discloses the claimed invention with the exception of the fluidized bed treatment, and the drying and storing step of claim 19. Weitzen et al. discloses contacting a liquid to be treated with a sorbent material in a fluidized bed; and it would have been obvious to one of ordinary skill in the art at the time the invention was made to contact the brine and magnetite of Curtain et al. in the manner suggested by Weitzen et al., in order to promote contact between the magnetite and brine of this primary reference system and/or to prevent clogging of the sorbent bed in this primary reference system. Such modification of the primary reference will inherently result in the formation of an upper zone comprising a complex of crystalline metallic ore and fat soluble compound, and a lower zone comprising crystalline metallic ore particles, for substantially the same reason that Applicant's recited process produces such upper and lower zones. Also, delaying removal of the sorbed constituent from the sorbent material in the manner recited in

Art Unit: 1724

claim 19 is not seen to materially affect the overall results of the reference process, or to produce any new and unexpected result; and is therefore deemed to be an obvious matter of choice, dependent upon when it is desired to recover the separated material, insufficient to patentably distinguish this claim.

Applicant's arguments filed June 13, 2003 have been noted and carefully considered but are not deemed to be persuasive of patentability. Applicant argues that "CURTAIN et al. and WEITZEN et al. both fail to disclose or suggest that magnetite can absorb or adsorb beta carotene" and that "both publications fail to teach that the density of the magnetite will decrease upon doing so." It is pointed out, however, that since both Applicant's recited process and the reference process contact the same stream (i.e. an aqueous brine solution containing *Dunaliella salina*) with the same treatment material (i.e. magnetite), the results obtained in both of these processes must inherently also be the same. It is axiomatic that one who performs the steps of a process must, in so doing, necessarily produce all of its results, for these results naturally flow from it, and are an inseparable part of it. Mere recitation of a newly discovered function or property that is inherently possessed by things in the prior art does not cause a claim drawn to those things to distinguish over the prior art. *General Electric. Co. v Jewel Incandescent Lamp Co.*, 67 USPQ 155 (1945); *In re Oelrich*, 212 USPQ 323 (C.C.P.A. 1981); *In re Best*, 195 USPQ 430 (C.C.P.A. 1977); *In re Swinehart*, 169 USPQ 226 (C.C.P.A. 1971).

Applicant also argues that Curtain et al. states that their process is applicable only to brines with a salt concentration of at least 3M, whereas Applicant's process works at salt concentrations of 1M, as shown in example 1. Again, this argument has been noted and

Art Unit: 1724

carefully considered, but is not deemed to be persuasive of patentability, particularly since none of the claims recite any salt concentration.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to I. Cintins whose telephone number is (703) 308-3840. The examiner can normally be reached on Monday through Friday from 8:30 AM to 5:00 PM. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mr. Blaine Copenheaver, can be reached at (703) 308-1261.

The fax phone numbers for this art unit are: (703) 872-9311 for "Official" faxes after Final Rejection; (703) 872-9310 for all other "Official" faxes; and (703) 872-9492 for "Draft" and other "Unofficial" faxes.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0661.



**Ivars C. Cintins**  
**Primary Examiner**  
**Art Unit 1724**

I. Cintins  
September 15, 2003

**(19) World Intellectual Property Organization  
International Bureau**



**(43) International Publication Date**  
**19 July 2001 (19.07.2001)**

**PCT**

**(10) International Publication Number**  
**WO 01/51162 A1**

**(51) International Patent Classification<sup>7</sup>: B01D 15/02, B01J 8/20, C07C 403/24**

(74) Agents: PIPER, James, William et al.; Pipers, 16 Byron Avenue, Takapuna, P.O. Box 33-1153, North Shore City, Auckland (NZ).

**(21) International Application Number:** PCT/NZ00/00001

**(22) International Filing Date:** 10 January 2000 (10.01.2000)

**(25) Filing Language:** English

**(26) Publication Language:** English

**(81) Designated States (national):** AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW.

**(71) Applicants (for all designated States except US): PIPER, James, William [NZ/NZ]; 90 Bassett Road, Remuera, Auckland 1005 (NZ). BETA NUTRITION LIMITED [AU/AU]; 21 Cumming Road, Oakford, W.A. 6205 (AU).**

**(84) Designated States (regional):** ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**(72) Inventor; and**

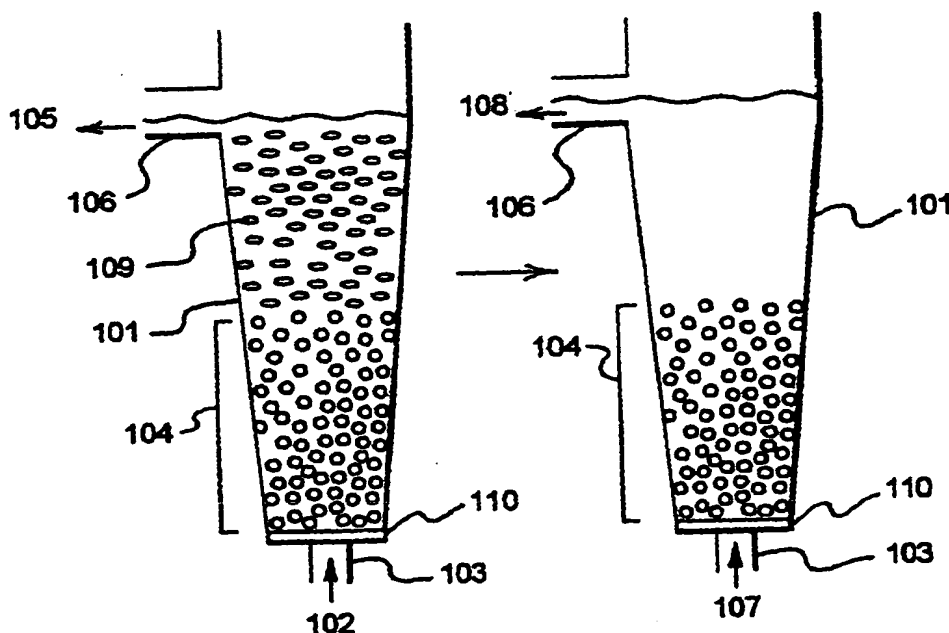
(75) **Inventor/Applicant (for US only):** **KEATING, Peter, James [AU/AU]; 14 Hart Road, Serpentine, W.A. 6125 (AU).**

**Published:**

— with international search report

*[Continued on next page]*

**(54) Title:** A METHOD FOR RECOVERING PIGMENTS FROM ALGAL CULTURES



**(57) Abstract:** A method and apparatus (101) for the recovery of fat soluble compounds, such as beta carotene, is described. In one embodiment of the invention a solution (102) containing a fat soluble compound is passed through a fluidised bed (104) of crystalline metallic ore particles, such as magnetite, allowing the fat soluble compound to bind to the particles to form a complex (109). The fat soluble compound is released from the complex (109) by passing a wash solution (107) through the fluidised bed and subsequently collected in solution (108). The crystalline metallic ore particles may be reused.

**WO 01/51162 A1**

WO 01/51162 A1



*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

## ***A METHOD FOR RECOVERING PIGMENTS FROM ALGAL CULTURES***

### **Field**

This invention relates to a method of recovering fat soluble compounds, including but not restricted to pigments such as beta-carotene, from solutions, including but not restricted to those solutions containing microalgal cells.

### **Background**

Intensive cultivation of microalgal cells is widely used as the source of a range of biological materials produced by algae including lipids, pigments and protein. A major limitation to the commercial feasibility of manufacturing such materials using algal biotechnology is the fact that microalgal cells exist at relatively low concentrations in water, are of very small size and can be mechanically and osmotically fragile. The harvesting of algal cells and their products at a commercial scale requires processes which concentrate the small algal cells and their constituent chemical products in an efficient manner which is simple, reliable and requires minimal energy inputs.

To date, methods which have been developed involve using either energy requiring processes such as centrifugation and drying, or use low energy processes such as flocculation, settling or algal behavioural responses which are unreliable and inefficient. Other methods require the disintegration of the algal cells which can render any cellular components useless; for example, degradation of valuable components, such as the carotenoids, via oxidation can occur.

One example of a method which may be used to obtain certain cellular components of algal cells, without any adverse degradation of those cellular components, is described in the patent specification relating to PCT/AU82/00165 entitled "Method for Harvesting Algae". This specification concentrates on methods for harvesting and concentrating algae, including *Dunaliella*, from suspensions of a certain salinity, whereby the whole algal cells are adsorbed

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onto an appropriate adsorbent media. The principle finding relating to this invention is that algal cell membranes become hydrophobic at salt concentrations above 3M enabling them to adsorb onto substances having a hydrophobic surface. A number of suitable hydrophobic adsorbents are described in this specification. In addition, a process of rendering certain  
5 adsorbents hydrophobic, or more hydrophobic, by treatment with silanes for example is described.

In PCT/AU82/00165 the whole-cell-adsorbent-media complex is then processed using organic solvents which damage the cell membrane and potentially which allow cellular components, such as beta-carotene, to be released while the cellular debris and insoluble cell components  
10 remain adsorbed to the adsorbent media. The beta-carotene released into the organic solvent in this invention may contain contaminants such as triterpenoids and other lipids and thus further processing is required to isolate only the beta-carotene.

### Object

It is an object of the present invention to provide an improved method of extracting fat-soluble  
15 compounds or at least to provide the public with a useful choice.

### Statement of Invention

In one aspect of the present invention there is provided a method of extracting fat-soluble compounds from aqueous solutions including the steps:  
providing an aqueous solution in which a fat-soluble compound is present;  
20 providing a bed of crystalline metallic ore particles held in an appropriate vessel;  
applying the aqueous solution to the bed of crystalline metallic ore particles substantially near the bottom of the bed at a rate sufficient to form and maintain a fluidised bed of crystalline metallic ore particles;  
allowing the fat-soluble compound to attach to the crystalline metallic ore particles to form a  
25 crystalline-metallic-ore-fat-soluble-compound complex;  
providing a wash solution;  
contacting the wash solution with the crystalline-metallic-ore-fat-soluble-compound complex to desorb the fat-soluble compound from the complex;



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collecting the wash solution containing the fat-soluble compound; and  
isolating the fat-soluble compound from the wash solution.

Preferably the crystalline metallic ore particles are magnetite particles.

5 Preferably the wash solution is contacted with the crystalline-metallic-ore-fat-soluble-compound complex by applying the wash solution to the fluidised bed of crystalline metallic ore particles substantially near the bottom of the fluidised bed and at a rate sufficient to maintain the bed in a fluidised state and the resultant wash solution containing the fat-soluble compound is collected from near the top of, or above, the fluidised bed of crystalline metallic ore particles.

10 Preferably the method further includes the step of collecting the crystalline-metallic-ore-fat-soluble-compound complex prior to providing a wash solution and contacting the wash solution with the crystalline-metallic-ore-fat-soluble-compound complex.

Preferably the crystalline-metallic-ore-fat-soluble-compound complex is collected from a region substantially near the top of the fluidised bed of crystalline metallic ore particles by means of continuous decantation.

15 Preferably the crystalline-metallic-ore-fat-soluble-compound complex is dried and stored for a period prior to being contacted with the wash solution.

Preferably the fat-soluble compound is present in the aqueous solution within a number of cells and the aqueous solution is a culture media.

Preferably the cells are those of *Dunaliella salina*.

20 Preferably the fat-soluble compound is a natural pigment.

Preferably the pigment is a carotenoid.

Preferably the carotenoid is beta-carotene.

Preferably the wash solution is an organic solvent.

Preferably the fat-soluble compound is isolated from the wash solution by evaporation or drying.

In another aspect of the present invention there is provided a substantially pure fat-soluble compound obtained using the method of any one of claims 1 to 13.

- 5 In yet another aspect of the present invention there is provided a crystalline-metallic-ore-fat-soluble-compound complex obtained using a method as herein described.

### Figures

These and other aspects of the present invention, which should be considered in all its novel aspects, will become apparent from the following description of the preferred embodiment of  
10 the invention, which are given by way of example only, with reference to the accompanying figure in which:

Figure 1 illustrates a preferred extraction apparatus and method according to the present invention; and

15 Figure 2 illustrates a preferred extraction apparatus and alternative method according to the present invention.

### Preferred Embodiment

The preferred embodiment of the invention is described below in terms of the recovery of beta-carotene from water containing the microalgal species *Dunaliella salina* (*D.salina*). It will be appreciated by those of general skill in the art that the invention would be applicable to the  
20 recovery of the other carotenoids and to other fat-soluble pigments from *D.salina*, and to the recovery of carotenoids or other fat-soluble compounds or pigments from other suitable organisms. The process of the present invention may also be applicable to the extraction from an aqueous solution of fat-soluble compounds suspended therein.

Throughout the following description the words adsorption and absorption, or derivatives thereof, such as adsorb or absorb, are used. The word adsorb is used to describe how a substance can be held on the surface of another and the word absorb to refer to the inclusion or incorporation of one substance into another. These words have been used interchangeably in the following text as the interactions between the substances (beta-carotene and magnetite) may be referred to in either way. In addition, the word attach is used to cover both adsorption and absorption. Those of general skill in the art will appreciate this factor.

#### General Principles of the Preferred Embodiment of the Invention

Beta-carotene is one of a group of compounds called carotenoids; this group also includes alpha carotene, lutein, lutein monoepoxide, astaxanthin, zeaxanthin, canthaxanthin, and lycopene. These compounds are well characterised and those skilled in the art will recognise them as being coloured fat-soluble compounds which function as part of the light-capturing apparatus in photosynthetic pathways. Beta-carotene, in particular, is a precursor to vitamin A, a vitamin obtained from dietary sources, rather than *de novo*, in animals. In addition, the carotenoid family have been associated with antioxidant activities. As a result, carotenoids and in particular, beta-carotene, are sought after for use in many food and health products.

The microalgae *Dunaliella* are typically cultivated in water which has a high concentration of dissolved salts, particularly concentrates of seawater such as those used for the production of salt by solar evaporation. Such waters are very corrosive of metal which they come into contact with. Under conditions of optimal nutrient concentrations, moderate temperatures and intense solar radiation, *Dunaliella* can grow to concentrations of one million algal cells per ml. The individual cells can contain up to 10% of their weight as beta-carotene, and thus beta-carotene can accumulate to the extent of 15 mg per litre of brine. The rest of *Dunaliella* cell biomass is composed of protein, carbohydrates and other lipids.

25 The process of the present invention uses an absorption medium, magnetite, which absorbs beta-carotene with very high affinity, but does not absorb significant amounts of the other components of the cell mass. However, beta-carotene is a lipid which is contained within the cell membrane, as opposed to being secreted from the cell and thus free in solution, therefore

*Dunaliella* cell membranes must be disrupted before the beta-carotene is available for absorption.

Magnetite is a crystalline iron ore which has a surface of sharp edges with numerous cracks, crags and irregularities. It has been identified that in the present invention magnetite can be  
5 used for the dual purpose of disrupting cell membranes and absorbing beta-carotene. When *Dunaliella* cells are brought into contact with magnetite particles the cell membrane is punctured by the numerous sharp edges and the cell contents are disgorged into the bulk growth/culture medium.

Another hitherto undescribed property of magnetite is that, because of its unique structure, it  
10 selectively absorbs beta-carotene. This occurs as beta-carotene, being a lipid, is insoluble in water and the surface of magnetite crystals are somewhat hydrophobic in nature. When beta-carotene is present in an environment of brine and magnetite, it partitions towards the more hydrophobic solid phase than the hydrophilic liquid phase. When the surface of crags within the magnetite particle become coated in beta carotene, a more hydrophobic microenvironment is  
15 created in which further beta carotene is absorbed. The total loading of beta-carotene into magnetite is thus very high; for example 2% to 4% of the mass of the magnetite. At this concentration, the void spaces within the magnetite structures are filled with beta-carotene. It will be understood by those of general skill in the art that it will not always be appropriate to load the magnetite completely as it may impact on the downstream recovery of beta-carotene.

20 Yet another useful property of magnetite disclosed herein is that when beta-carotene is adsorbed onto, then absorbed into magnetite, the oxidation processes which ordinarily cause the rapid decay of beta-carotene, particularly when it is exposed to oxygen, are inhibited such that the magnetite/beta-carotene complex is very stable and does not degrade when exposed to heat or when it is dried.

25 Because of the above identified properties of magnetite which are not obvious, magnetite provides an ideal material upon which to collect and concentrate beta-carotene. However, it will be appreciated by those of general skill in the art that alternative absorption media, such as

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another crystalline metallic ore which has properties equivalent to those of magnetite, for example hematite, may be used in the process of the present invention.

As indicated above when beta carotene has been absorbed on magnetite until the magnetite is saturated, the material is, for example, around 2% by weight beta-carotene. As the brine  
5 containing *D. salina* typically has a maximum beta-carotene concentration of 20 parts per million, absorption by the magnetite, in this example, thus concentrates the beta-carotene by a factor of one thousand fold. Magnetite typically has a bulk density of 4 Kg per litre whereas the brine used for growing beta-carotene-containing *D. salina* has a bulk density typically of 1.2 Kg per litre. Therefore by passing 1,000 litres of brine containing *D. salina* through one kilogram  
10 of magnetite, all the beta-carotene can be removed and contained in a volume of 250 ml, a concentration of almost 4,000 fold.

The beta-carotene can be easily desorbed from the magnetite by simply washing the magnetite with a suitable wash solution such as an organic solvent. Both polar and non-polar solvents are suitable for this purpose. Ordinarily non-polar solvents would not easily mix with a material  
15 such as magnetite when it is wetted with water or brine due to hydrophobicity. However, another useful feature of the microcrystalline structure of magnetite is that interfacial tension is broken by the sharp surface, thus a non-polar solvent is easily able to penetrate, and then dewater the magnetite.

The organic solvent used to desorb beta-carotene will contain essentially pure beta carotene, as  
20 other microalgal products are not absorbed onto the magnetite. Because the solvent contains pure beta-carotene it is particularly easy to remove the beta carotene and recover the solvent for re-use, for example by using reduced pressure devices such as crystallisers.

Solvents which are suitable for desorbing beta-carotene from magnetite include, but are not restricted to acetone, ethanol, hexane, petroleum ether, or any mixtures of these solvent.  
25 Further, due to consumer demand for natural products it is preferable that natural solvents be used. In this regard we have found terpene alcohols to be efficient solvents for use in this invention; for example, cineol (eucalyptus oil), *d*-limonene (lemon oil), citral (citrus oil) and terpen-4-ol (tee tree oil).

Basic Apparatus and Extraction Example of the Preferred Embodiment of the Invention

An example of an apparatus in which the process of the preferred form of the invention may be conducted is shown in Diagram/Figure 1 and Figure 2. In Figure 1 the magnetite is contained within a conical vessel (101). The magnetite sits on a distribution plate, or plenum, (110) which separates the inlet pipe (103) from the vessel interior. This plenum (110) allows a solid phase of magnetite to settle onto the bottom of the vessel when the apparatus is not in use.

Brine containing *D. Salina* (102) is introduced at the bottom of the conical vessel via inlet pipe (103) at such a flow rate as to maintain the magnetite as a fluidised bed (104). Being a fluidised bed contactor, there is no prospect for the adsorption media, magnetite, to become clogged. The brine (containing cellular debris) (105) leaves the vessel at outlet pipe (106). It can either be sent into another similar vessel to (101) if it still contains unabsorbed Beta-carotene, or it can be returned to the algal growth pond.

One can see from Figure 1 that the fluidised bed separates into two different layers or phases. The bottom phase contains primarily magnetite particles and the top phase magnetite-beta-carotene complexes (109); which move upwards because of a change in their density due to forming the complex with beta-carotene. It will be appreciated by those of general skill in the art that the layers may not be distinct from one another as illustrated in Figures 1 and 2 and that while two layers do form they do so across a gradient as a result of the degree of loading of magnetite with beta-carotene. Further, it will be appreciated that the size of the magnetite particle and the velocity of fluid flowing into the vessel will have an effect on the position of that particle within the vessel. The Figures have been simplified to illustrate that magnetite loaded with beta-carotene will decrease in density during the process.

It should be noted that magnetite of various particle sizes may be used in the present invention. The size of such particles is not important in relation to the absorption of beta-carotene but it will have an effect on the behaviour of the fluidised bed (104). Thus, as a result of the particle size of the magnetite used the flow rate of solutions into the vessel (101) may be required to be altered to maintain the bed (104) in a fluidised state.

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The beta-carotene can be desorbed from the magnetite in the top phase by changing the flow into the inlet at (103) from brine to the desorption solvent (107) of choice. A different flow rate is required to keep the magnetised bed fluidised due to the density differences between brine and the solvent. The solvent effluent (108) from (106) contains essentially pure beta-carotene which  
5 can be recovered by evaporating the solvent. During this stage magnetite present in the top phase may fall to the bottom phase as the beta-carotene is released and its density increases.

It can be clearly seen that by using a number of vessels such as (101), connected in series such that the outlet (106) of one is connected to the inlet (103) of the next vessel in the series, any number of vessels can be connected to each other. If both the inlet and the outlet are connected  
10 via a manifold which can feed either brine or desorption solvent into the vessel, then a continuous process cycle of adsorption/desorption/adsorption is possible.

Such a system operates at low pressure, has only valves as moving parts, can be constructed of cheap plastic material and has a very low energy requirement. As such, this system provides a very simple, efficient and reliable means of harvesting beta-carotene from brine.

15 Figure 2 uses the same apparatus as Figure 1 but illustrates an alternative embodiment in which when the magnetite has become progressively loaded with beta-carotene the magnetite-beta-carotene complex (109) is collected from the vessel (101) at outlet (106). At this stage the complex can be washed immediately with an appropriate solvent or stored at room temperature for prolonged periods without any significant deterioration of the contained beta-carotene and  
20 washed at a later date.

These basic examples will become further apparent from the specific examples 1 to 3 which follow.

#### Specific Examples relating to the Preferred Embodiment of the Invention

##### Example 1:

25 A culture of *Dunaliella salina* was grown in outdoor ponds containing sodium chloride at a concentration of 60g per litre (approximately 1 M). When the culture had attained a beta-carotene concentration of 9mg per litre, the culture was pumped into the bottom of a vertical

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perspex cylinder of 100mm diameter at a rate of 1.5 litre per minute. When the cylinder became filled with liquid, 800g of magnetite (120 mesh) was introduced into the top of the cylinder.

The magnetic moved towards the bottom of the cylinder but became suspended within the cylinder as a fluidised bed which maintained a height of 400mm. When the fluidised bed became stable, the culture which passed through the bed to the top of the cylinder was sampled and the beta carotene concentration was measured and found to be 3.9mg per litre.

While the culture medium was still being pumped through the bottom of the cylinder, a further 400g of magnetite was then introduced into the top of the cylinder. The fluidised bed then expanded to a height of 580mm. The culture emerging from the top of the cylinder was again sampled and this time found to have a beta carotene concentration of 1.9mg per litre.

A further 400g of magnetite was then added to the cylinder which caused the fluidised bed height to increase to 750mm. At this bed height the culture emerging from the top of the bed appeared clear. The beta carotene concentration was measured and found to be 0.04mg per litre.

After approximately 1 hour of operation with a total added volume of magnetite of 1,600g, and a constant upward culture medium flow rate of 1.5 litres per minute, the fluidised bed volume had expanded to 780mm, and had separated into two distinct zones. The upper zone had a slightly red colour and was 65mm high. The lower zone was the same black colour as the originally formed fluidised bed and was 715mm high. There was a distinct boundary between the two layers.

Magnetite material from the upper layer was collected using a pipette, then washed with fresh water and examined under a microscope. There was no sign of any algal cells adhering to this magnetite. The magnetite was then dried in a flow of warm air, weighed accurately and the washed with acetone. The acetone was collected and the beta carotene concentration in the acetone was determined by measuring the optical density at 450nm wavelength. It was determined in this way that the magnetite contained 3.9% by weight beta carotene.

#### Example 2:

A culture of *Dunaliella salina* was grown in outdoor ponds containing sodium chloride at a



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concentration of 60 g per litre (approximately 1 M) and magnesium chloride at 60g per litre (approximately 1 M). When the culture had attained a beta carotene concentration of 11 mg per litre, the culture was pumped into the bottom of a vertical perspex cylinder of 100mm diameter at a rate of 1.4 litre per minute. When the cylinder became filled with liquid, 1.600g of magnetite (120 mesh) was introduced into the top of the cylinder. The magnetite moved towards the bottom of the cylinder but became suspended within the cylinder as a fluidised bed which maintained a height of 800 mm. When the fluidised bed became stable, the culture which passed through the bed to the top of the cylinder was sampled and the beta carotene concentration was measured and found to be 0.07mg per litre. The culture medium emerging from the top of the column was examined under a microscope. There were no intact algal cells observed, however cellular debris, comprising mostly broken cell membranes, and halobacteria were observed.

After approximately 2 hour of operation at a constant upward culture medium flow rate of 1.4 litres per minute, the fluidised bed volume had expanded to 845mm, and had separated into two distinct zones. The upper zone had a slightly red colour and was 165mm high. At this height the magnetic fluidised bed had reached the top of the perspex cylinder. As the bed expanded further, the top layer spilled over and was collected and was examined under a microscope. There was no sign of any algal cells adhering to this magnetite. The magnetite was then dried in a flow of warm air, weighed accurately and then washed with acetone. The acetone was collected and the beta carotene concentration in the acetone was determined by measuring the optical density at 450nm wavelength. In this way it was determined that the magnetite contained 3.8% by weight beta carotene.

### Example 3:

A culture of *Dunaliella salina* was grown in outdoor ponds containing sodium chloride at a concentration of 90g per litre (approximately 1.5 M) and magnesium chloride at 90g per litre (approximately 1.5 M). When the culture had attained a beta carotene concentration of 14mg per litre, the culture was pumped into the bottom of a vertical perspex cylinder of 100mm diameter at a rate of 1.65 litre per minute. When the cylinder became filled with liquid, 1.600g of magnetite (120 mesh) was introduced into the top of the cylinder. The magnetic moved

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towards the bottom of the cylinder but became suspended within the cylinder as a fluidised bed which maintained a height of 800mm. When the fluidised bed became stable, the culture which passed through the bed to the top of the cylinder was sampled and the beta carotene concentration was measured and found to be 0.06mg per litre.

- 5 In this example the cylinder was modified by creating a spillway 950mm up the length of the cylinder. After 95 minutes of operation, the upper (red) zone of the fluidised magnetite bed had reached the spillway, and magnetite began trickling from the spillway. This spilled magnetite was separated from the culture medium by decantation. The rate of flow of magnetite trickling from the cylinder was estimated by collecting the material for one minute, removing the culture  
10 medium by decantation and weighing the magnetite. It was found that approximately 600mg of magnetite was spilling from the cylinder each minute. By washing the magnetite with acetone and measuring the optical density of the washing acetone at 450nm, the spilled magnetite was found to contain 3.65% by weight beta carotene.

- For the next 4 hours a 6g sample of fresh magnetite was added to the top of the cylinder every  
15 10 minutes. The fresh magnetite could be seen to travel through the upper red zone into the lower black zone of the fluidised bed. For the 4 hours during which the trial was undertaken, the fluidised bed maintained a more-or-less constant height and a quite constant rate of red magnetite spillage from the spillway. At the completion of the trial, 400 litres of culture medium had been passed through the cylinder and substantially all the beta-carotene had been  
20 removed.

- The fluid flow into the cylinder was then switched from culture medium at 1.65 litres per minute to cineole at a flow rate of 2.25 litres per minute. Again a fluidised bed was formed, this time with a bed height of 820mm. The cineole emerging from the top of the cylinder was a deep red colour. Spectrophotometric measurement of the cineole at 450nm showed it contained beta  
25 carotene at a concentration of 1.35% w/v. After about four minutes of flow, the cineole emerging from the top of the cylinder became a paler red, and after 6 minutes it was clear. All the eluted cineole was collected, and evaporated using a rotary evaporator. As the cineol evaporated, dark crystals of beta carotene were formed. When the cineole had completely

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evaporated, the remaining crystalline material was collected and weighed. The material weighed 5.44g.

It can be concluded that the 400 litres of culture originally applied to the magnetite fluidised bed contained 5.6g of beta carotene. Of this, 5.44g was recovered in crystalline form from the  
5 cineole eluent. This represents a recovery of over 97% of the original beta carotene present in the culture.

### **Industrial Application and Advantages**

Carotenoids and other fat-soluble pigments are sought after additives for food and health products. The process of the present invention is very simple, requires little more energy than  
10 that needed to reticulate water containing microalgae to the apparatus and thus provides an efficient means of extracting these compounds from their source and thus may prove of commercial and economic advantage.

The process has the further advantage of stabilising the product and enabling the convenient storage of the product as a concentrate.

**CLAIMS:**

1. A method of extracting fat-soluble compounds from aqueous solutions including the steps:  
providing an aqueous solution in which a fat-soluble compound is present;  
providing a bed of crystalline metallic ore particles held in an appropriate vessel;  
5 applying the aqueous solution to the bed of crystalline metallic ore particles substantially near the bottom of the bed at a rate sufficient to form and maintain a fluidised bed of crystalline metallic ore particles;  
allowing the fat-soluble compound to attach to the crystalline metallic ore particles to form a crystalline-metallic-ore-fat-soluble-compound complex;  
10 providing a wash solution;  
contacting the wash solution with the crystalline-metallic-ore-fat-soluble-compound complex to desorb the fat-soluble compound from the complex;  
collecting the wash solution containing the fat-soluble compound; and  
isolating the fat-soluble compound from the wash solution.
- 15 2. A method as claimed in claim 1 wherein the crystalline metallic ore particles are magnetite particles.
3. A method as claimed in any one of claims 1 or 2 wherein the fat-soluble compounds attach to the metallic iron ore particles via means of adsorption or absorption.
4. A method as claimed in any one of claims 1 to 3 wherein the wash solution is contacted with  
20 the crystalline-metallic-ore-fat-soluble-compound complex by applying the wash solution to the fluidised bed of crystalline metallic ore particles substantially near the bottom of the fluidised bed and at a rate sufficient to maintain the bed in a fluidised state and the resultant wash solution containing the fat-soluble compound is collected from near the top of, or  
above, the fluidised bed of crystalline metallic ore particles.
- 25 5. A method as claimed in any one of claims 1 to 3 further including the step of collecting the crystalline-metallic-ore-fat-soluble-compound complex prior to providing a wash solution

and contacting the wash solution with the crystalline-metallic-ore-fat-soluble-compound complex.

6. A method as claimed in claim 5 wherein the crystalline-metallic-ore-fat-soluble-compound complex is collected from a region substantially near the top of the fluidised bed of crystalline metallic ore particles by means of continuous decantation.
7. A method as claimed in any one of claims 5 to 6 wherein the crystalline-metallic-ore-fat-soluble-compound complex is dried and stored for a period prior to being contacted with the wash solution.
8. A method as claimed in claim any one of claims 1 to 7 wherein the fat-soluble compound is present in the aqueous solution within a number of cells and the aqueous solution is a culture media.
9. A method as claimed in claim 8 wherein the cells are those of *Dunaliella salina*.
10. A method as claimed in any one of claims 1 to 9 wherein the fat-soluble compound is a natural pigment.
11. A method as claimed in claim 9 wherein the pigment is a carotenoid.
12. A method as claimed in claim 11 wherein the carotenoid is beta-carotene.
13. A substantially pure fat-soluble compound obtained using the method of any one of claims 1 to 12.
14. A crystalline-metallic-ore-fat-soluble-compound complex obtained using the method of claim 7.

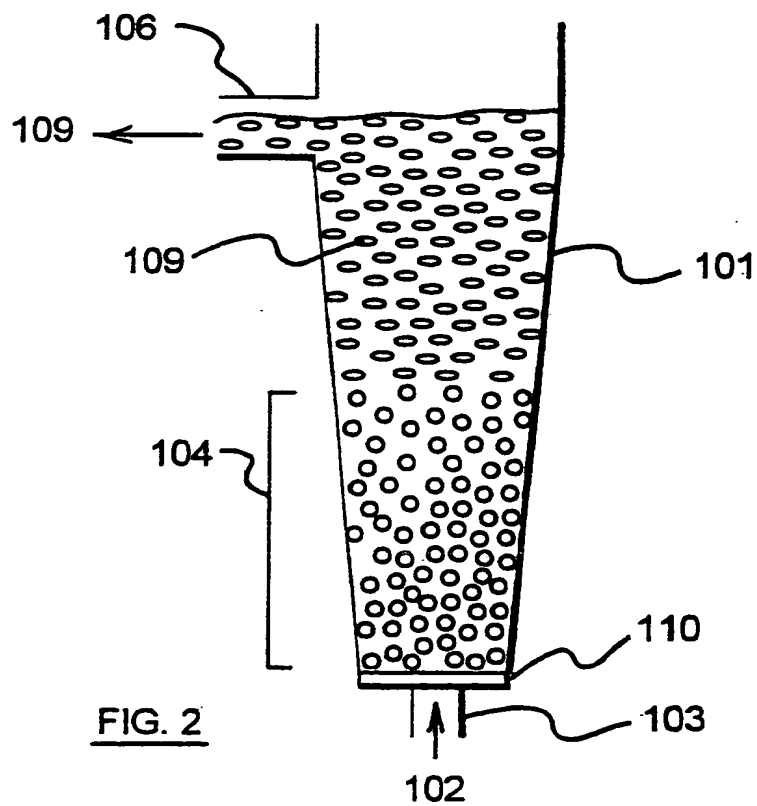
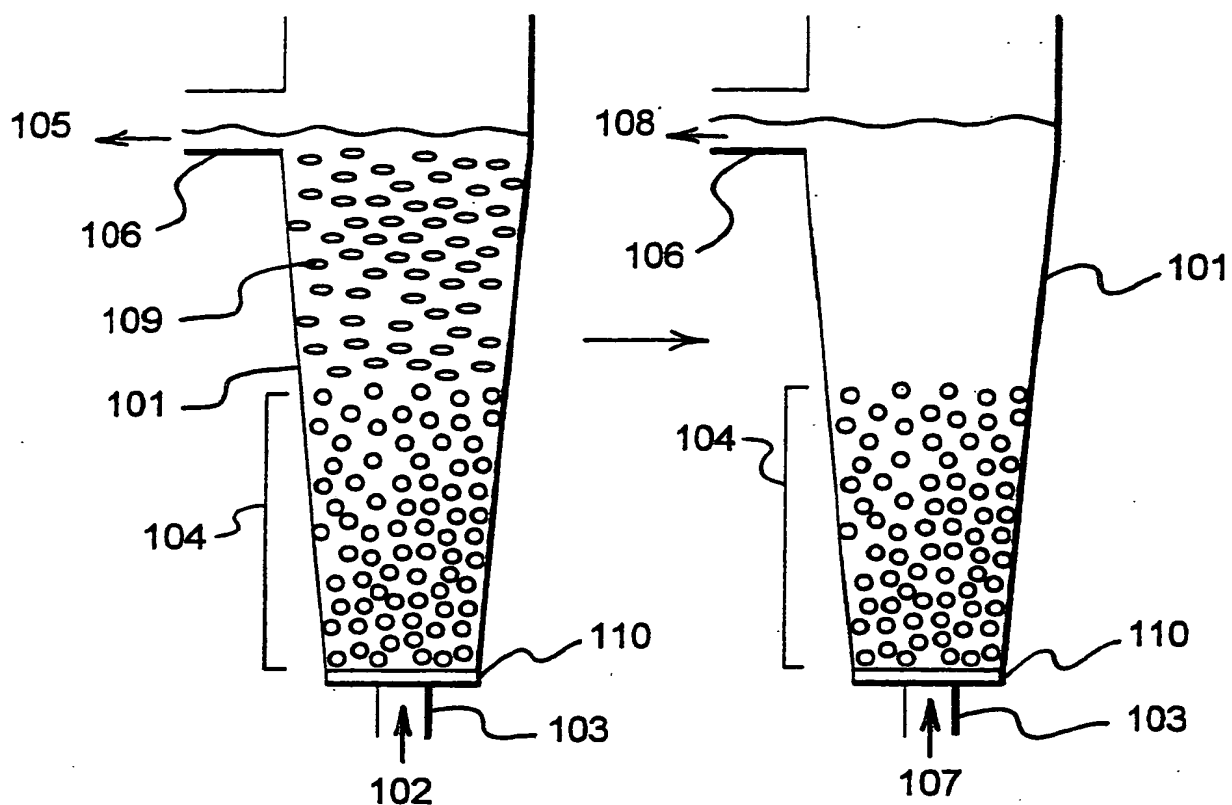


FIG. 2

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/NZ 00/00001

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>												
Int Cl <sup>7</sup> : B01D 15/02 B01J 8/20 C07C 403/24												
According to International Patent Classification (IPC) or to both national classification and IPC												
<b>B. FIELDS SEARCHED</b>												
Minimum documentation searched (classification system followed by classification symbols)												
B01D 15/02 B01J 8/20, C07C 403/24												
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched												
RU IPC as above												
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)												
WPAT:MAGNET+												
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>												
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.										
A	WO 92/198237 A (PHARMACIA LKB BIOTECHNOLOGY AB)29/10.92											
A	US 4284511 A (WEITZEN) 18.08.81											
A	DE 19638004 A (SKW TROSTBERG AG)											
<input type="checkbox"/> Further documents are listed in the continuation of Box C <input checked="" type="checkbox"/> See patent family annex												
<p>* Special categories of cited documents:</p> <table border="0"> <tr> <td>"A" Document defining the general state of the art which is not considered to be of particular relevance</td> <td>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"E" earlier application or patent but published on or after the international filing date</td> <td>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td>"&amp;" document member of the same patent family</td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			"A" Document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family	"P" document published prior to the international filing date but later than the priority date claimed	
"A" Document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention											
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone											
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art											
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family											
"P" document published prior to the international filing date but later than the priority date claimed												
Date of the actual completion of the international search 30 April 2000		Date of mailing of the international search report 10 MAY 2000										
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA E-mail address: pct@ipaustalia.gov.au Facsimile No.: (02) 6285 3929		Authorized officer  G. CARTER Telephone No.: (02) 6283 2154										

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**PCT/NZ 00/00001**

Patent Document Cited in Search Report				Patent Family Member	
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